

ON CERTAIN NEW METHODS OF BLOOD EXAMINATION

WITH SOME INDICATIONS OF THEIR CLINICAL
IMPORTANCE

BY

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ON CERTAIN NEW METHODS OF BLOOD EXAMINATION WITH SOME INDICA- TIONS OF THEIR CLINICAL IMPORTANCE.

AN exhaustive examination of the blood is impossible with the present methods of collecting blood from the patient. When the blood is collected into tubes or capsules and is allowed to clot there the serum alone becomes available for examination. When blood films only are prepared we are confined to a histological examination of the corpuscular elements. When, lastly, blood is drawn off into a diluting fluid for the enumeration of the corpuscles we are tied down to this particular examination. These limitations can be transcended by drawing off the blood into a calibrated capsule charged with a measured quantity of citrate of soda solution. The blood thus prevented from clotting furnishes for our examination all the different blood-elements: red corpuscles, white corpuscles, blood-platelets, and plasma. The red corpuscles are furnished in a form in which they can be employed indifferently for histological examination and for quantitative estimation. Even more than this is achieved in the case of the white corpuscles. These are furnished in a form which admits not only of their histological examination and enumeration but also of the measurement of the phagocytic power which accrues to them in the plasma or as the case may be in any other medium which may have been substituted for this.

Method of collecting blood into a calibrated capsule charged with a measured volume of citrate of soda solution.—The capsule with recurved limb already figured by me in previous papers¹ is the form of capsule best adapted to the

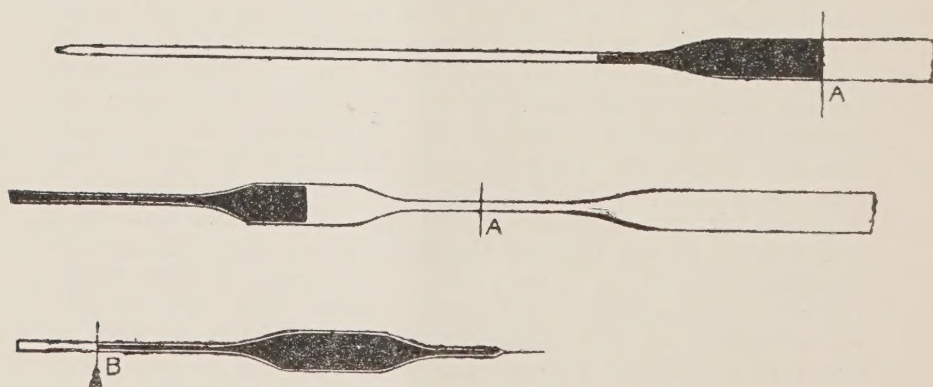
¹ Brit. Med. Jour., Feb. 5th, 1898; Proceedings of the Royal Society 1902; THE LANCET, July 25th, 1903, p. 214.

procedure described below. For the purpose here in question it is charged with a quantum of citrate of soda such as suffices to keep the blood which is received into it liquid. The capsule is further furnished with a fiduciary mark to indicate the point to which it is to be filled in with blood. The calibration of the capsule and the filling in of the measured charge of citrate of soda solution can be accurately and expeditiously carried out by the aid of a pair of automatic pipettes. The respective cubic capacities of these are so adjusted as to stand to each other in the quantitative relation which is to obtain between the blood and the citrate of soda solution.

Method of making the automatic pipettes.—The smaller of the two automatic pipettes is made as described in a previous paper in THE LANCET.² The procedure is varied only in the respect that 50 cubic millimetres, more or less, in lieu of 5 cubic millimetres, of mercury are employed and in the respect that the orifice of the pipette is in the course of construction drawn out into a point in the flame of a peep light. The larger automatic pipette which serves for calibrating the blood capsule is made in the following way.

A piece of glass tubing is taken in hand. It is drawn out into a roomy capillary stem and this is again drawn out into a sharp point. By the aid of the smaller automatic pipette a volume of mercury corresponding approximately to 300 cubic millimetres and accurately to six fills of the smaller pipette is introduced into the neck of the tube. The mercury is allowed to pass down some little distance into the capillary stem, as shown in Fig. 1. A fiduciary mark (A, Fig. 1) is

FIGS. 1, 2, 3.

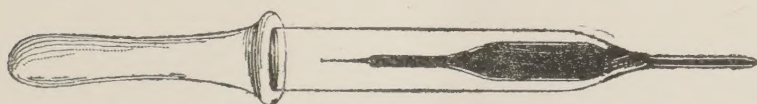


now placed upon the barrel of the tube to mark the upper limit of the mercury. This done the mercury is allowed to run down into the capillary stem so as to leave vacant the upper end of the tube in the region of the fiduciary mark. The tube is now introduced into the blow-pipe flame and is drawn out in such a manner as to bring the fiduciary mark into the position indicated by A in Fig. 2. The narrow tube thus obtained is by the aid of the flame of a peep light

² Thrombosis in Typhoid Fever, THE LANCET, Dec. 6th, 1902, p. 1531.

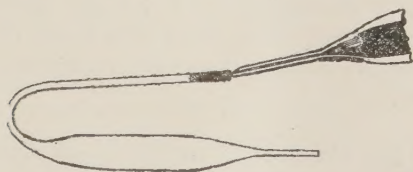
drawn out at the fiduciary mark into a tube of such fineness as to be impervious to mercury while remaining patent to air. This done the glass is allowed to cool down. The next step is to bring back the mercury into the bulb of the pipette, as shown in Fig. 3, and to place a mark B upon the capillary stem at the lower limit of the mercury column. The measuring tube is completed by drawing out the capillary stem at the point indicated by the mark B and breaking it off there so as to obtain a pointed extremity. A thick layer of sealing-wax is now applied to the neck of the bulb. This effected, a covering tube is prepared by drawing out a piece of wider tubing and breaking it across just below the neck. Into this covering tube we insert the measuring tube from above and draw the capillary stem down through the neck. Finally, we apply heat in such a manner as to melt the sealing-wax and to make an air-tight joint (Fig. 4).

FIG. 4.



Method of calibrating the blood capsule and charging it with the citrate of soda solution.—Having broken off the points of both the recurved and of the straight limbs of the blood capsule the larger automatic pipette is taken in hand. Placing a rubber teat over the upper end and making pressure upon this a negative pressure is established in the interior. The orifice of the automatic pipette is now inserted into a reservoir of mercury and the metal is allowed to flow in until the bulb is full. When this has been accomplished the point of the pipette is withdrawn from the mercury and is brought in contact with the orifice of the recurved limb of the blood capsule (Fig. 5). By pressing upon the teat the

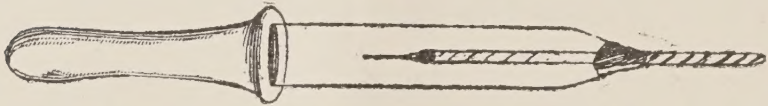
FIG. 5.



mercury is in an almost automatic manner transferred to the capsule. The mercury in the blood capsule is shaken together and the capsule is tilted in such a manner as to bring a column of mercury back to the neighbourhood of the orifice through which it was introduced. The height at which the mercury is standing in the recurved limb and in the barrel of the capsule is now marked off with a glass writing pencil. The smaller automatic pipette is now taken in hand (Fig. 6). A negative pressure having been established in its interior a small bead of mercury is drawn into the orifice of the tube. The point of the pipette is then transferred

from the vessel of mercury to a vessel containing a 4 per cent. citrate of soda solution made up in physiological salt solution. On relaxing the pressure on the teat the pipette sucks up the citrate of soda solution until the bead of mercury which is carried up in front of the column of fluid arrives at the terminal hair-fine tube and there establishes a block which prevents any further intake (Fig. 6). The

FIG. 6.



point of the pipette is now applied to the orifice of the recurved limb, pressure is made on to the teat, and the watery fluid transfers itself to the capsule in an even more automatic manner than was the case with the mercury. The charged capsule may either be immediately employed or its ends may be sealed and it may be put aside till required.

Method of filling in blood from the finger.—The extremities of the capsule having been broken off a prick is made in the side of the finger with a hypodermic needle. A handkerchief is then wound round the digit from above downwards in such a manner as to render the extremity of the finger definitely turgid. Pressure is now made upon the pulp and the blood as it emerges from the prick is received into the recurved limb of the capsule to commingle there with the citrate of soda solution. When the blood has reached the fiduciary mark the upper end of the capsule is warmed in such a manner as to rarefy the contained air. The upper limb is then immediately sealed up. As soon as the glass has cooled and the contraction of the air has drawn up the blood from the recurved limb into the body of the capsule, this last is shaken to effect thorough mixture of the citrate of soda and blood.

Appearance of the blood as it settles down in the capsule.—The advantage of the method begins to exhibit itself within a very few minutes. Figs. 7, 8, 9, which are taken from actual specimens, exhibit the appearances of three different bloods. Fig. 8 represents a blood drawn from a patient suffering from Bright's disease. The volume of the corpuscular sediment is approximately normal. The plasma has a milky aspect. Fig. 7 represents blood taken from a patient the subject of extreme anæmia.³ The characteristic feature of the blood is the small volume of the corpuscular sediment. Fig. 9 represents blood drawn from

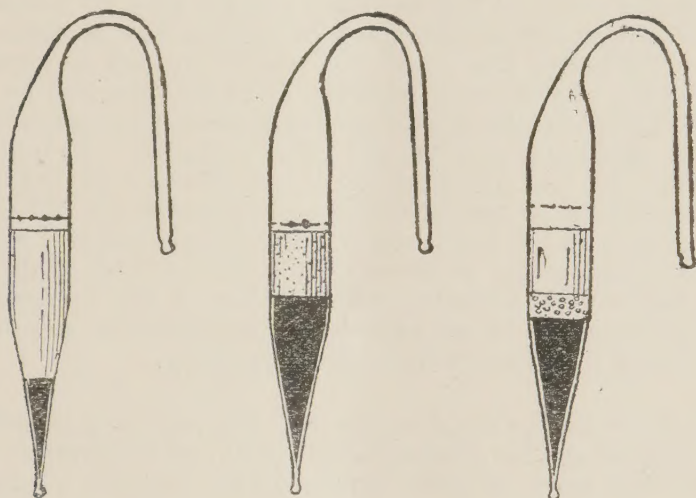
³ The number of red corpuscles in this patient's blood, as determined by the hæmocytometer, was 650,000 per cubic millimetre.

a leucocythæmic patient.⁴ The exaggerated depth of the layer of white corpuscles is the salient feature. A noticeable feature also is the reduction in the red corpuscles. Equally characteristic is the appearance of the blood in cases of jaundice. Here, and the same holds true also in some cases where active hæmolysis is going on, the plasma is definitely yellow.

FIG. 7.

FIG. 8.

FIG. 9.



Further procedures.—The further procedures may be undertaken at any convenient time after the withdrawal of the blood. Opportunity is thus afforded for the conveyance of blood to the laboratory or, if need arises, for its transmission by the post. In the following I will confine myself to describing certain new developments in connexion with the methods of blood examination.

Quantitative estimation of the corpuscular elements.—The defects of the hæmocytemetric method are well understood. They are summed up in the fact that the results of a blood count may deviate from the truth to practically an unlimited extent if the calibration of the apparatus is at fault or if the operator is either unwary or wanting in skill. A further regrettable feature in the hæmocytemetric method is the unsuitability of the method for purposes of demonstration. The enumerator alone can take personal cognisance of the results. The only alternative which at present offers is the measurement of the corpuscles by the hæmokrit. While the principle of this method has met with general approval its

⁴ The number of leucocytes in this patient's blood—as determined by the hæmocytemeter—was just over 500,000 per cubic millimetre. The diagnosis of leucocythæmia was arrived at in the course of a few minutes by the appearance of the blood as it settled in the capsule.

particular application in the hæmokrit is open to objection on the ground that it involves recourse to a highly-gearred centrifuge and manipulation of somewhat delicate apparatus. In point of fact, nothing of this kind is necessary. The volumetric measurement of the corpuscles can be carried out in a perfectly effective manner by the simple procedure of filling in citrated blood into a capillary tube which has been marked off into a series of divisions of equal cubic capacity. In the case where the result of the measurement is required with the minimum of delay the graduated stem of the capillary pipette may be broken off short at the neck and the hand centrifuge may be called into use. Ordinarily the blood may simply be left to sediment overnight. By the next morning sedimentation will be practically complete and the number of volume divisions occupied by the corpuscles can be read off at a glance. As indicated in Figs. 11 and 12 the number of volumes of corpuscles corresponding to ten divisions of the undiluted blood may range from one or below one in cases of very extreme anæmia to six divisions (or a little less) in the normal adult male. The following is a method which allows of the division of a capillary pipette in an expeditious manner into any desired number of divisions of equal cubic capacity.

Instructions for dividing the stem of a capillary pipette into any desired number of divisions of equal cubic capacity.—Take in hand a piece of glass tubing drawn out into a capillary stem and draw it out below into a fine point. The capillary pipette thus obtained may be employed just as it is, or it may if desired be formed into a throttled pipette similar to the pipettes shown in Figs. 10 to 13. In any case fit a tightly fitting rubber teat upon the upper end of the pipette and inscribe a mark upon the stem at a distance from the orifice corresponding to about one-twelfth of its total length. Making pressure on the teat and holding the pipette at a considerable angle to the vertical thrust the pointed end below the surface of some mercury which has been placed in a watch-glass and which has been covered in with water. Now let the mercury run up and fill the tube up to the fiduciary mark A, Fig. 10, then push the point of the pipette out through the mercury into the water and let a similar volume of this last pass up into the tube. Withdrawing again the point of the pipette under the cover of the mercury, go through the whole manœuvre again until the tube has, as is shown in Fig. 10 been filled in with a series of six alternate volumes of water and mercury. This done withdraw the point of the pipette from the watch-glass and mark off with a pencil a series of six divisions. Having marked off these six (which will be all that will be required for the measurement of the corpuscular sediment), bring the series of six alternate volumes of mercury and water further up into the tube, and taking off in this measurement from the

FIG. 10.

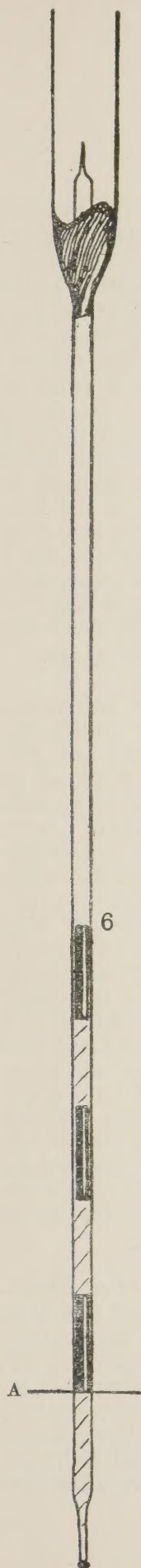


FIG. 11.



FIG. 12.

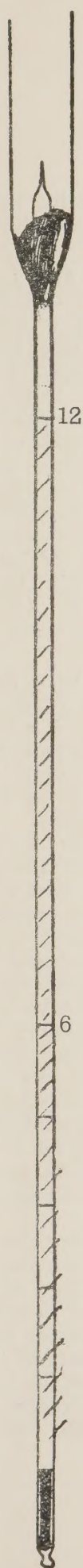


FIG. 13.



sixth division inscribe upon the upper end of the capillary stem a fiduciary mark corresponding to the twelfth division.

Instructions for filling in the graduated capillary pipette with citrated blood.—Shake up first the contents of the blood capsule in such a way as to bring into suspension the corpuscles which may have settled down. Then fitting a teat to the graduated pipette draw up into it from the capsule exactly 12 volumes of citrated blood. Dealing as we are with a five-sixths dilution of the blood these 12 divisions will correspond to 10 of the undiluted blood. After rectifying the position of the column of blood in the tube, seal up the pointed extremity of the tube in the flame of a peep light and—unless in the case where the centrifuge is to be employed—place the capillary pipette upright in a rack. Figs. 11, 12, and 13 represent the appearances furnished by filling in this manner graduated capillary pipettes respectively with normal blood and with blood from a case of extreme anæmia and a case of Bright's disease.

Preparation of blood films from corpuscular sediment.—When we desire to proceed to the histological examination of the corpuscles we may with advantage make our films either from the corpuscular sediment generally or as the case may be from a particular stratum of the corpuscular sediment, diluting in each case with such amount of plasma as may be convenient. This method of procedure enables us even in the case of the most extreme anæmias—where this is by the ordinary technique impossible—to concentrate in the microscopic field a sufficiently large number of the corpuscular elements to make an effective histological examination. The procedure promises further advantages, for judging from the fact that it is possible by diluting the blood with an equal bulk of physiological salt solution to obtain the white corpuscles separate from the red it will probably be found possible by an appropriate dilution of the blood to bring together into a particular stratum of the sediment the particular elements which happen to constitute the object of our search. By this process of levigation it may, for instance, prove possible to bring together, as the case may be, red corpuscles charged with malaria parasites, nucleated red corpuscles, and free living parasitic elements of all kinds.

Method of preparing slides for the reception of blood films.—In connexion with the histological examination of the blood passing reference may appropriately be made to, perhaps, the most important factor involved in the preparation of a blood film. This factor is the preliminary treatment of the slide. Popular prejudice to the contrary notwithstanding, it will be found that the really important thing in connexion with the slide is to secure that its surface shall be rough. The polishing of the surface and the defective adherence

between the blood and the slide which results from it furnish the opportunity for the piling up of the corpuscles one upon the other under the influence of the surface tension of the fluid. The polishing of the slide is, in other words, directly responsible for the patchiness of the ordinary blood film. I find it to be a wise procedure to boil all slides in strong caustic potash until their surface has become definitely hazy. It is hardly necessary to point out that such haziness, dependent as it is upon inequalities of the surface, altogether disappears when the oil or, as the case may be, the Canada balsam is placed upon the slide.

Estimation of the quantity of blood platelets.—Milkeness of the blood fluids is a characteristic feature of the blood in many cases of diabetes and Bright's disease. It is met with also as a temporary and less conspicuous phenomenon in the healthy in the course of active digestion. This condition of the blood—it is commonly misnamed lipæmia—appears in point of fact to depend upon an extraordinary excess of blood platelets.⁵ Two points of interest with regard to these may be noted—first, that blood which contains platelets in quantities sufficient to render the plasma absolutely milky is not more coagulable than ordinary blood; and secondly, that it may be possible—and this has been suggested to me by my friend, Captain Stewart R. Douglas, I.M.S.—to proceed to the enumeration of the platelets in citrated plasma. This suggestion has its justification in the circumstance that the platelets do not in the case of citrated blood run together into clumps and they remain in suspension indefinitely after the settlement of the corpuscles.

Quantitative estimation of the amount of albuminous substance in the plasma or serum.—We shall remain in ignorance with respect to the causes of many œdemas and with regard to the proper method of treating these conditions just so long as we neglect in dealing with these disorders to investigate the character of the circulating blood fluid and of the lymph. What is perhaps most urgently required for such an investigation is a method of determining the content of the blood in albuminous substances. Until a better method shall have been elaborated the following will, I think, render useful service.

Principle of the method.—The method here proposed aims at the determination of the degree of dilution to which the blood may be subjected before it loses the property of coagulating into a stiff jelly when subjected to a temperature just below that of boiling water. The apparatus required is a capillary tube, a little mercury, water at a temperature

⁵ The identification of the particulate matter in milky plasmas with blood platelets is warranted by the characteristic staining reaction obtained with Leishman's stain.

just below boiling point, and a glass-cutting knife, or in default of this a pair of small bone forceps. The material subjected to examination may be any sufficiently concentrated albuminous fluid. In particular it may be the plasma, serum, blister fluid, or pericardial, pleuritic, or ascitic effusion.

Procedure.—The first step in the procedure is to make an appropriate series of dilutions of the fluid. In dealing with blood I have commonly employed in addition to the undiluted serum dilutions of $\frac{5}{6}$ ths, $\frac{4}{6}$ ths, $\frac{3}{6}$ ths, $\frac{2}{6}$ ths, and $\frac{1}{6}$ th. A series consisting of undiluted serum and in association with this $\frac{7}{8}$ ths, $\frac{6}{8}$ ths, $\frac{5}{8}$ ths, $\frac{4}{8}$ ths, and $\frac{3}{8}$ ths dilutions of serum would be more appropriate.

The dilutions first spoken of are made as follows. The stem of a capillary pipette is, by the method already described above, divided into six equal divisions. By the aid of a rubber teat this tube is filled with serum up to the fifth division. One volume of physiological salt solution is then aspirated into the tube and the whole is blown out and mixed upon a glass slide. Four volumes of this mixture are re-aspirated into the tube and an additional volume of diluting fluid is added. This furnishes a dilution of $\frac{4}{6}$ ths. After it has been mixed upon the slide three volumes of the mixture are re-aspirated and again an additional volume of salt solution is added, furnishing a $\frac{3}{6}$ ths dilution. Of this, two volumes are re-aspirated into the tube and a third volume of diluting fluid is added. This furnishes a $\frac{2}{6}$ ths dilution. Lastly one volume of this dilution is re-aspirated and it is diluted with one volume of salt solution, furnishing a dilution of $\frac{1}{6}$ th.

The alternative series of dilutions above referred to would be prepared either on exactly the same principle as above or as follows. By taking three volumes of serum and adding one of diluting fluid, then taking two of the $\frac{4}{6}$ ths serum and one of diluting fluid, and lastly, by taking one of $\frac{3}{6}$ ths serum and one of diluting fluid we obtain a series of dilutions corresponding to $\frac{3}{6}$ ths, $\frac{2}{6}$ ths, and $\frac{1}{6}$ th serum. Taking this preliminary series of dilutions and, in addition, undiluted serum we proceed to obtain the intermediate dilutions as follows. Mixture of equal volumes of undiluted serum and $\frac{3}{6}$ ths serum would give us $\frac{4}{6}$ ths serum, mixture of equal volumes of $\frac{3}{6}$ ths serum and $\frac{2}{6}$ ths serum would give us $\frac{5}{6}$ ths serum, and mixture of equal volumes of $\frac{2}{6}$ ths serum and $\frac{1}{6}$ th serum would give us $\frac{3}{6}$ ths serum.

Having obtained the appropriate series of dilutions our next step is to draw up these progressive dilutions into a capillary inspissation tube and to separate them off one from the other. Our graduated tube may do service as our inspissation tube or we may employ for this purpose any other capillary tube which has been drawn out at its extremity into a sharp point. In either case we proceed as follows. We draw up first one volume of the highest dilution, then carefully avoiding, here and at all subsequent stages of the procedure, the intrusion of bubbles of air we draw into the point of our pipette a pellet of mercury of sufficient size to divide off securely from the next dilution of serum. We in this way take up into the tube the whole series of dilutions of serum, dividing off in each case by mercury. This process accomplished we seal the point of the tube in the flame, taking care as we do so not to include any appreciable quantity of air. We now plunge our in-

spissation tube into a vessel of water which has just begun to cool down after having been brought to the boil. After waiting from three to four minutes we withdraw our tube from the water and fit to its upper end a rubber teat in the collapsed condition. Leaving the negative pressure thus generated to do its work uncontrolled and taking the tube into one hand and a bone forceps into the other we proceed to snip off the point of the pipette. By so doing we ascertain whether the clot which has been formed in the undiluted serum is or is not competent to resist the pressure of the atmosphere. If it holds fast we snip away the capillary stem piece by piece. As our successive lines of section traverse the successive mercury pellets we take note in each case that we are passing from one dilution of the serum to the other. Finally, we arrive at the point where the coagula proximal to the line of section yield to the negative pressure. When this happens and the column of clotted fluid is carried up into the expanded end of the pipette we take note of the fact that we have exceeded the limits of dilution in which the serum when coagulated by heat offers effectual resistance to the pressure of the atmosphere. Tested by the above method the blood of normal male adults gives a resistant clot in a maximum dilution of four-sixths to three-sixths.

Remarks on the above method.—While the method described above makes no pretence to absolute accuracy it will be found, when put to the test, by making a series of successive estimations of any sample of blood, to yield very concordant results within the range of calibre which is dictated, on the one hand, by considerations of the amount of serum ordinarily available and, on the other hand, by the limit imposed by the considerations that the capillary tube must not be so fine as to offer sensible resistance to the passage of fluids nor be so weak as to be incapable of resisting ordinary strains. In connexion with the clinical application of the method it may be interesting here to call attention to the information which was furnished by the method in a case of cedema of the feet which was when first seen supposed to be referable to double femoral thrombosis supervening upon typhoid fever. In this case the method of examination here in question revealed that the content of the blood in albuminous substances was reduced to the point at which the clot formed by heating the undiluted serum was quite incompetent to resist the pressure of the atmosphere. The same held true of the lymph obtained by the application of a blister. In view of the unlikelihood of thrombosis occurring under such conditions, and further, in view of the negative result of Widal's test, the treatment by citric acid which had been inaugurated with a view to limiting the extension of the presumed clot was abandoned and magnesium sulphate was administered with a view to concentrating the blood. Under this treatment the cedema rapidly disappeared and the serum yielded on heating a resistant clot.

Estimation of the content of the plasma in fibrinogen.—In concluding this paper reference may be made to the circumstance that an estimate of the amount of fibrinogen in the plasma can be formed by the very simple device of heating citrated plasma in a tube up to a temperature of 60° C. The plasma in hæmophilia when examined by this method yields, as compared with the normal plasma, a notably diminished volume of fibrinogen.

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